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Protective effect of Cortisone and Hydrocortisone drugs on lysosomal damages induced by bacterial endotoxin in wistar rats

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Objectives: Bacterial endotoxin as biological stress by multiple organs failure causes lysosomal enzyme leakage. Lysosome as a basic cytoplasmic organelle in animal tissues contains hydrolytic enzymes capable of degrading various cellular constituents. In this study protective effect of Cortisone acetate and hydrocortisone 21-sodium hemisuccinate on lysosomal damage and its association with change level of serum and hepatic acid phosphatase activity investigated. **Methods:** In this study, 30 rats equally divided to Control, tolerance and Endotoxin groups. The tolerance group (12.5 mg/kg body weight intramuscularly injection Cortisone acetate for 3 days and on the 4th day, the intravenous injection 12.5 mg/kg of hydrocortisone 21-sodium hemisuccinate). The induce endotoxin shock in rats with 2.5 mg/kg body weight intravenous injection of Salmonella endotoxin. Partial purification and beta-glucuronidase activity were determined by sephadexG75 chromatography and Polyacrylamide Gel Electrophoresis. **Results:** The results of this study shown a significant different in level serum and homogenate acid phosphatase activity in Tolerance group compared with the other groups ($P<0.05$). Also enzyme especial activity in all steps of purification, in Endotoxin group was more than the other groups ($P<0.05$). **Conclusion:** Endotoxin shock as biological stressor by induction of lysosomal enzymes into the cell plays an important role in deterioration of cells. Also, it seems that protection of these particles by injection of cortisone acetate and hydrocortisone 21-sodium hemisuccinate can a significant resistance to induced stress by endotoxin shock.

INTRODUCTION

Bacterial endotoxin as biological stressor lead to physiological responses in Animal and human (1-6). Lysosomes as cellular organelles sensitive to stress have a basic role in a collection of cellular processes (7-9), such as programmed cell death, autophagy, (6, 10-12) endocytosis, exocytosis, apoptosis (10, 13, 14), therefore bacterial endotoxin, high vitamin A (15-17) and irradiation by deteriorate membrane lysosomal cause releasing harmful enzymes (18, 19). So can say anoxia as an outcome of the event by disrupted in membrane lysosomes causes that hydrolase liberate into the cell. These enzymes by affect on cellular protein, nucleic acid, and polysaccharides significantly intensify the damages and help to expansion its irreversibility (18). Injection of bacterial endotoxin can elevates hydrolase lysosomal in

liver and muscle (19). The few doses of bacterial endotoxins can by deteriorate lysosome cause liberation of enzymes to large granule fraction liver (20) and glucocorticoids drugs partially reduced tissue damages by stabilizing the membrane lysosomal and inhibit release hydrolase (18). This study do in order to investigate effect of Cortisone acetate and Hydrocortisone sodium hemisuccinate on lysosome stability (21, 22). In this study acid phosphatase activity as biomarker stress enzyme estimated in serum and liver in three groups, also in order to determine effect cortisone and hydrocortisone drugs on the response of lysosomes to biological stress, partially purified enzyme be done. The present study, designed for the biochemical assay to determine the effects of bacterial endotoxin and glucocorticoid drugs inside the body.

MATERIALS & METHODS

Chemicals and Reagents

Salmonella enteritidis endotoxin, p-nitrophenylphosphate (pNPP), Cortisone Acetate and Hydrocortisone sodium hemisuccinate were purchased from Sigma Chemical Co.

Animals

In this study 30 male rats about 1.5 months old between 160 and 190 mg were used. The male rats were purchased from House animal of Ahvaz

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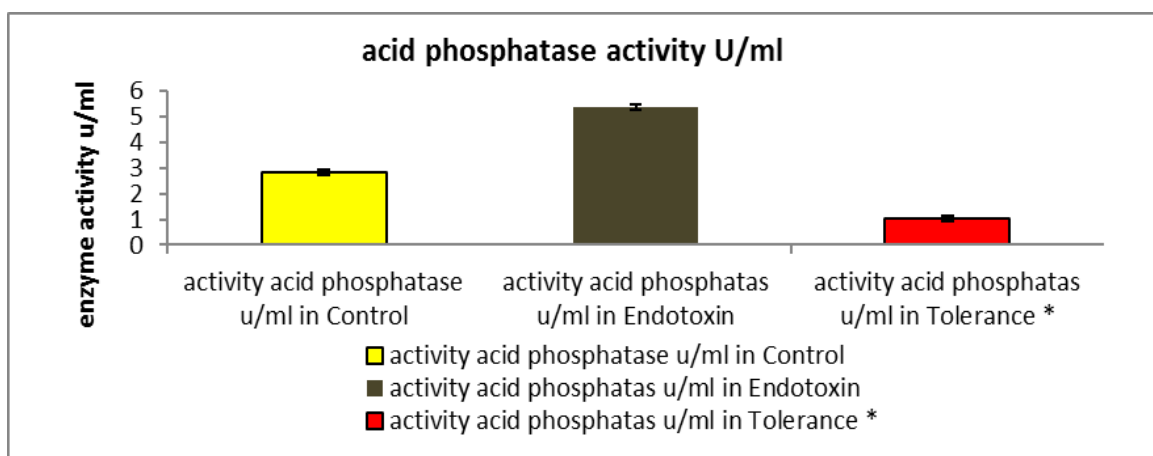


Figure 1 Determine level serum acid phosphatase activity in three groups Control, Tolerance, Endotoxin.

Jundishapur University of Medical Sciences, Ahvaz. All rats were maintained in standard condition with regular temperature control ($23 \pm 2^\circ\text{C}$), a 12:12 h light dark cycle, free access to water and food, and relative humidity of $55 \pm 5\%$.

Experimental design

These rats were equally divided into three groups ($n = 10/\text{group}$). Control group was treated with normal saline, the rats of tolerance group received Cortisone acetate (12.5 mg/kg, intramuscularly injection) for 3 days and on the 4th day they received hydrocortisone 21-sodium hemisuccinate (12.5 mg/kg, intravenous injection) and then an hour later Salmonella enteritidis endotoxin (2.5 mg/kg, intravenous injection) was injected (20), Endotoxin group (2.5 mg/kg, intravenous injection, Salmonella endotoxin). One hour afterward (23), sample blood taken from the ventricle left heart and serum was separated with centrifuged at 10000 g for 20 minutes at 40°C (24-26).

Preparation of a Liver Granular Fraction

Step 1: After separate liver, it was minced in ice and Sucrose (0.25 M) until the release of gross blood. **Step 2:** livers were weighed and homogenized at 1:10 w/v homogenate in sucrose (0.25 M) and 650 mL buffer at 900 rpm by Homogenizer device, for 5 minutes at 4°C . **Step 3:** The homogenate was centrifuged at 800 g for 10 minutes at 4°C , the supernatant was separated. **Step 4:** The supernatant was centrifuged at 15,000 g for 20 minutes, the supernatant was separated. **Step 5:** The supernatant of step four was again centrifuged at 15,000 g for 20 minutes. **Step 6:** The pellet of Step 5 was separated and homogenized in a glass Homogenizer than 1:5 w/v in sucrose for 5 minutes at 40°C and incubated at 37°C for 40 minutes. **Step 7:** The homogenate of Step 6 centrifuged at 15,000 g for 20 minutes and supernatants were assayed for the activities of lysosomal enzymes (15, 18, 20, 27, 28).

Partial Purification acid phosphatase Enzyme

Ammonium sulfate precipitation: The supernatant of step 7 was subjected to fractionation of ammonium sulfate precipitation. The acid phosphatase precipitated in a saturation range of 30–80% was centrifuged at 10,000 $\times g$ for 20 min. (29-31).

Gel Filtration Chromatography on Sephadex G-75: Dialyzed ammonium sulfate fraction was applied to the column of gel filtration Sephadex G-75 (32). Absorbance was read at 280 nm and beta-glucuronidase activity assay was read at 405 nm (33, 34). The liquid enzyme was concentrated to a volume of 5 mL by dialysis bag on

sucrose (32, 35, 36). Protein concentrations were determined by Bradford method (37).

Polyacrylamide Gel Electrophoresis: purity and homogeneity of the enzyme were show by electrophoresis in SDS-PAGE also protein migration on this gel indicated by Coomassie Brilliant blue or Silver staining (38, 39). Acid phosphatase activity was determined by King-Armstrong method and p-nitrophenylphosphate (pNPP) as substrate (40-43). Specific activity was expressed as U/mg protein and Unit of enzyme activity (44, 45).

Ethical principles

This article is a result of the research project of Ahvaz Jundishapur University of Medical Sciences with the code (IR.AJUMS.REC.1395.118) in student research committee of this University), (<http://proposal.ajums.ac.ir/>).

Statistical analyses

Statistical analysis was performed with SPSS version 18. Data was analyzed using descriptive statistical methods including mean and standard deviation, and compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant level was set at $P < 0.05$.

RESULTS

Comparison of serum level of acid phosphatase in Control, Tolerance and Endotoxin shock groups: We in this study indicate serum level of acid phosphatase after of induce bacterial endotoxin increase in the tolerance and endotoxin groups. But, in tolerance group was low enzyme activity due to resistance created by cortisone and hydrocortisone drugs. Figure 1 show average of enzyme activity in serum rats. This results shows that induced tolerance by Cortisone acetate and Hydrocortisone 21-sodium hemisuccinate decreases enzyme activity in serum.

Evaluation protein concentration and acid phosphatase activity of step, sephadex G75 Chromatography: This founds in figure 2 show a similar relationship between serum level acid phosphatase activity with liver homogenates. The data showed that there is significant difference in enzyme activity in suspensions obtained from liver in group and higher activity and concentration in group endotoxin than control and tolerance (Fig.2). Table 1 show the total protein and specific activity in various stages of purification enzyme in the three groups. Furthermore,

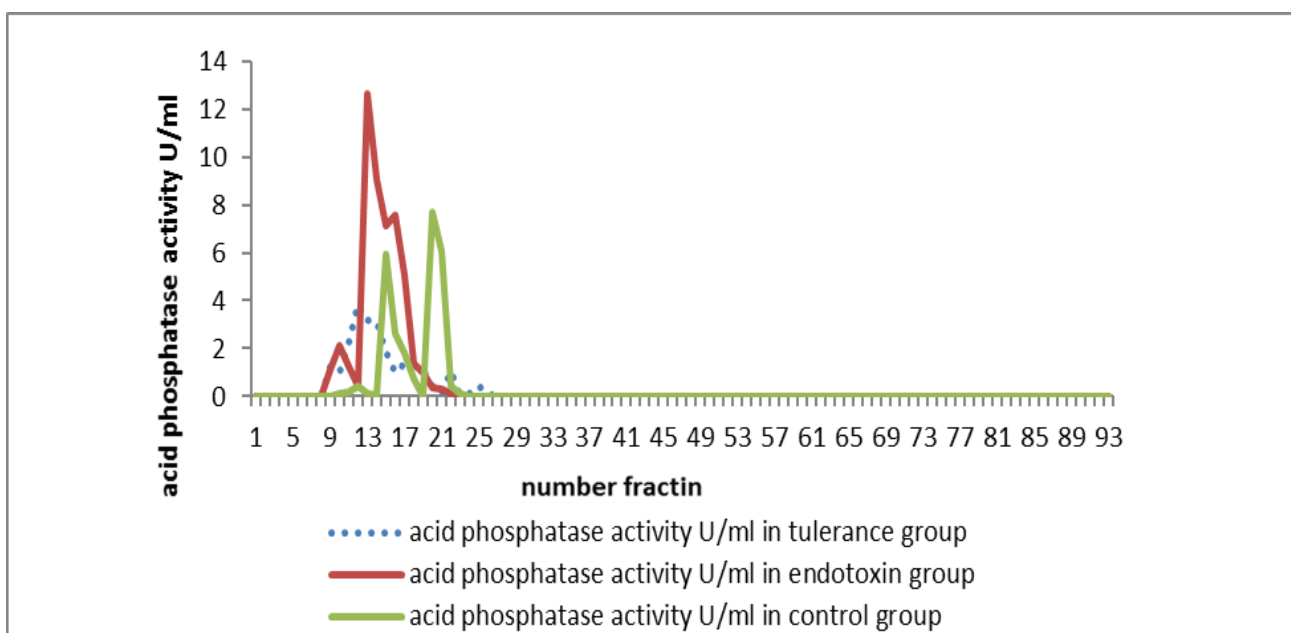


Figure 2 Comparison acid phosphatase activity in Control, Tolerance, Endotoxin groupse on sephadex G75.

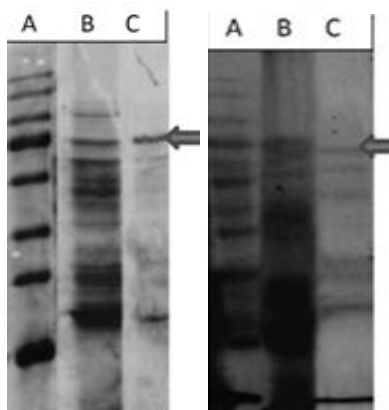


Figure 3 Endotoxin Group
Figure 4 Tolerance Group

Figure 3 & 4 Comparison polyacrylamide gel electrophoresis of the purified acid phosphatase enzyme from rat's liver in the Tolerance and Endotoxin group (A: ladder, B: Amoniumsulfate precipitate, C: Sephadex chromatography).

Table 1 Summary of pure acid phosphatase enzyme from rats liver Control, Endotoxin and Tolerance groups.

| Purification step | Groups | Vol.ml | Total units | Specific activity u/mg | Recovery % | Purification folds |
|------------------------------------|-----------|--------|-------------|------------------------|------------|--------------------|
| Saline extract | Control | 1000 | 9764.3 | 6.06735 | 100 | 1 |
| | Tolerance | 1000 | 7849.4 | 5.0735 | 100 | 1 |
| | Endotoxin | 1000 | 12816.9 | 10.65151 | 100 | 1 |
| Cantrificatio 15000 g3 | Control | 350 | 2422.1 | 5.995 | 24.805 | 0.688 |
| | Tolerance | 350 | 2209.7 | 4.9729 | 28.152 | 0.875 |
| | Endotoxin | 350 | 3271.5 | 6.850 | 25.525 | 0.929 |
| Amoniumsulfate precipitate | Control | 30 | 174.0 | 173.4353 | 1.782 | 37.226 |
| | Tolerance | 30 | 141.6 | 155.001 | 1.804 | 44.924 |
| | Endotoxin | 30 | 209.3 | 182.350 | 1.633 | 28.970 |
| Dialyzed ammonium sulfate fraction | Control | 25 | 101.4 | 175.714 | 1.038 | 36.833 |
| | Tolerance | 25 | 95.2 | 164.859 | 1.213 | 380261 |
| | Endotoxin | 25 | 142.2 | 190.137 | 1.109 | 40.487 |
| SephadexG-75 chromatography | Control | 20 | 36.0 | 260.124 | 0.368 | 45.280 |
| | Tolerance | 20 | 33.7 | 188.5751 | 0.429 | 52.365 |
| | Endotoxin | 20 | 53.0 | 294.933 | 0.414 | 58.340 |

the purity and homogeneity of the enzyme of sephadex G75 chromatography step as the latest step were checked by electrophoresis in SDS-PAGE and protein migration on this gel show by silver staining (37, 39). According to results of electrophoresis, the migration enzyme show as a single sharp band with a molecular weight of approximately 55 000 (KDa) by slab electrophoresis in three group control, tolerance (Fig. 4) and endotoxin shock (Fig. 3). Protein migration indicated a broader single band in the endotoxin group in comparing to control and tolerance, which its due to cell autolysis and more release lysosomal enzymes. So, this phenomenon approved increase enzyme specific activity in Endotoxin group and decrease specific activity in tolerance group (Table 1).

DISCUSSION

The various biochemical events are responsible for tissue injury, but little information was about cellular damages caused by bacterial endotoxin as biological stressor (46). The purpose of this study investigated protective effects Cortisone acetate and Hydrocortisone 21-sodium hemisuccinate on endotoxin induced lysosomal damages in wistar rats. This founds show that induced stress by bacterial endotoxin cause releases lysosomal enzymes in biological fluids (48). Present study shows bacterial endotoxin lead to significantly increase specific activity and protein concentration. On the other hand, induced tolerance by Cortisone acetate and Hydrocortisone 21-sodium hemisuccinate cause the lower liberation enzyme and the higher persistent in animals under acute biological stress. This founds from different steps purification enzyme (Table 1) indicated a considerable different and significant in total protein and enzyme activity in all steps purification in endotoxin group. it is Predicated that higher specific activity in endotoxin group is associated with autolysis cellular (48) and fragility membrane lysosomes, that its validity approved by earlier studies (46). This study approve attractive hypothesis Weissmann and Fell (47, 48), also Janoff theory (49). In addition, this result is consistent with previous studies do by Weismann, Gianetto, de Duve and Janoff that show endotoxin by enhancing permeability membrane influence lysosomes function and pretreatment glucocorticoids drugs can increase resistance to cell lysis and reduce intracellular activity (20, 23, 50). Dingle in studies on chondrocytes proteolytic activity noticed that Cortisone acetate and Hydrocortisone 21-sodium hemisuccinate postpone the action on cartilage matrix (15). Therefore, possibility Cortisone or Hydrocortisone drugs prevent of action stress on the tissue by inhibit hydrolase (51). Another hypothesis presented in this study was investigation purification enzyme and comparison of electrophoretic mobility of it on polyacrylamide gel. This distinct and broader band (in 55 KDa by using the marker protein or ladder) protein migration in endotoxin group is due to cell autolysis and lysosomal membrane disruption. Weight molecular acid phosphatase enzyme in papers was 55 KDa (52). Previous studies show increased enzymes activity as risk factors in Prostatic carcinoma, benign prostatic hypertrophy, prostatitis, multiple myeloma, Paget's disease, hyperparathyroidism, bone marrow metastases, sickle cell disease, thrombocytosis, lysosomal disorders, kidney disease, liver disease (cirrhosis), rape or aggression pathogenesis disease (52-55). But, relationship between biological stressor and enzymes requires further research on endocrine psychoanalysis (53). Therefore, this study could be considered as a controversial and new study, but unlike experimental variables, many of the biological variables are mutable, so we need to perform further studies on psychological and biological factors and their interaction with changes level enzymes under stress condition. Therefore, it seems that

pretreatment with Cortisone acetate and Hydrocortisone 21-sodium hemisuccinate will reduce the release of enzymes and its cellular degeneration.

CONCLUSION

The results showed that bacterial endotoxin as biological stress can affect stability of lysosomes and Cortisone or Hydrocortisone drugs can acts through the protection of these subcellular particles against a variety of injurious agents by decrease liberation and reduce activity of lysosomal enzymes.

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Conflict of interest

There are no conflicts of interest in this study.

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